



Studies on oral immunotherapy-induced immune changes at the local GI tissue through transcriptomics

Wenming Zhang¹, Gopal Krishna Ramadas Dhondalay¹, Ramona Hoh², Dana Tupa¹, Bryan J. Bunning¹, Nielsen Q. Fernandez-Becker¹, Neeraja Kambham², Scott Boyd^{1,2}, Stephen Galli^{1,2}, Sandra Andorf¹, Monali Manohar¹, R. Sharon Chinthrajah¹ & Kari Nadeau¹

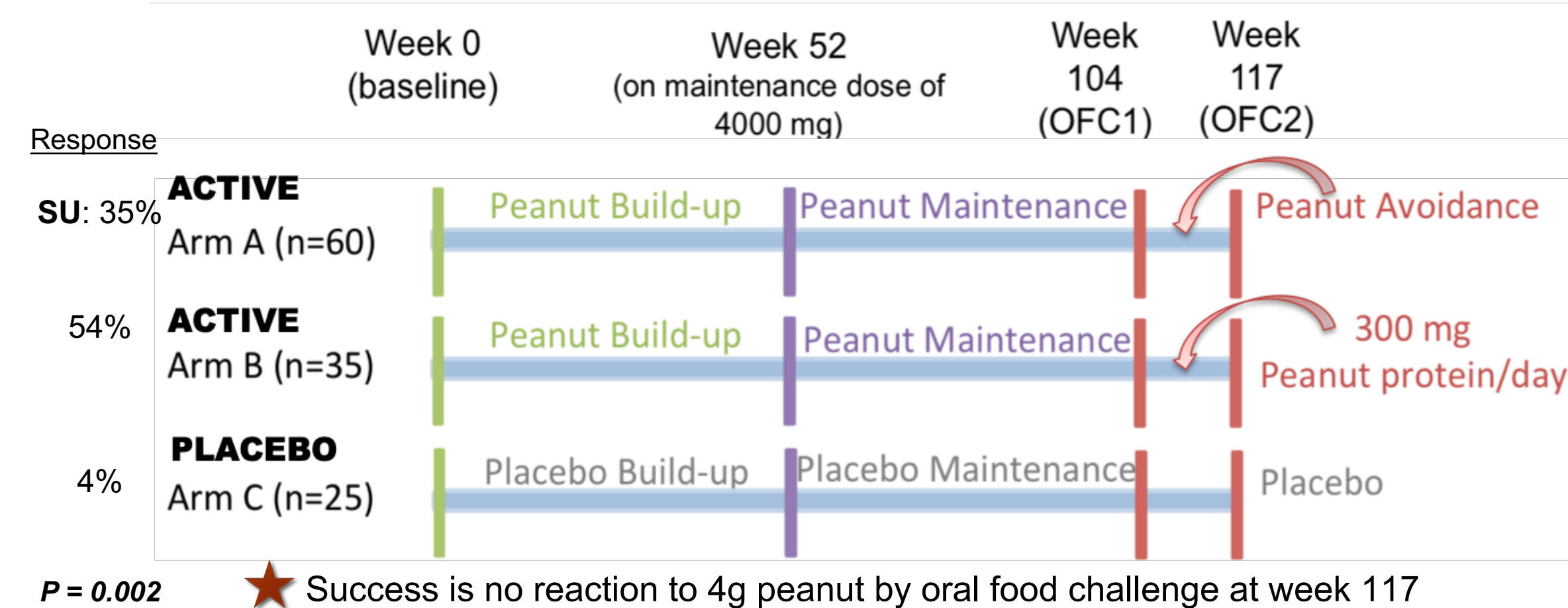
1. Sean N. Parker Center for Allergy & Asthma Research, Stanford University, Stanford, CA

2. Department of Pathology, Stanford University, Stanford, CA

BACKGROUND

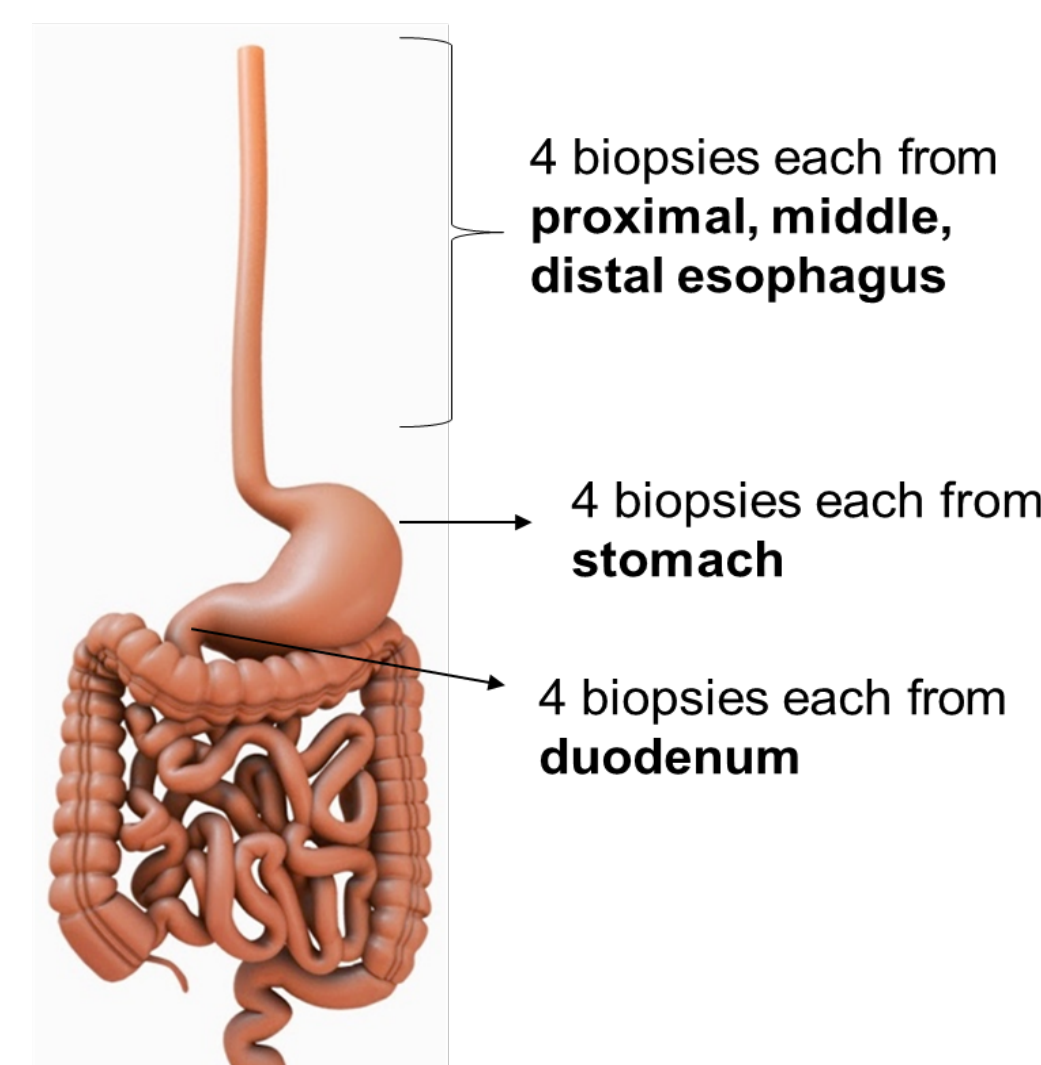
The findings from our phase 2, randomized, placebo-controlled peanut oral immunotherapy (OIT) trial (NCT02103270; POISED study) showed that peanut OIT could desensitize individuals with peanut allergy to 4000 mg peanut protein (Chinthrajah, RS, Lancet 2019, vol 394, P1437-1449).

Peanut Oral Immunotherapy : Safety, Efficacy and Discovery



While peripheral blood is the most accessible tissue to study the immune mechanisms behind food allergy and OIT, examining such mechanisms in the GI-resident immune cells will provide a far better, clinically relevant insight. We thus aimed to investigate immune changes in the biopsied GI tissue from a subset of participants from POISED study before and after peanut OIT using RNA-Seq.

SAMPLE COLLECTION



Twenty adults out of the 120 patients in POISED study into the GI sub-study in which patients underwent four GI endoscopy procedures.

Four biopsies were taken at each of the five tissue sites, proximal/middle/distal esophagus, stomach, and duodenum at each of the four time points viz. baseline, week 52, week 104, and week 117 into OIT.

METHODS

Bulk Tissue RNA-seq | RNA extraction, generation of cDNA libraries, sequencing, and data analysis

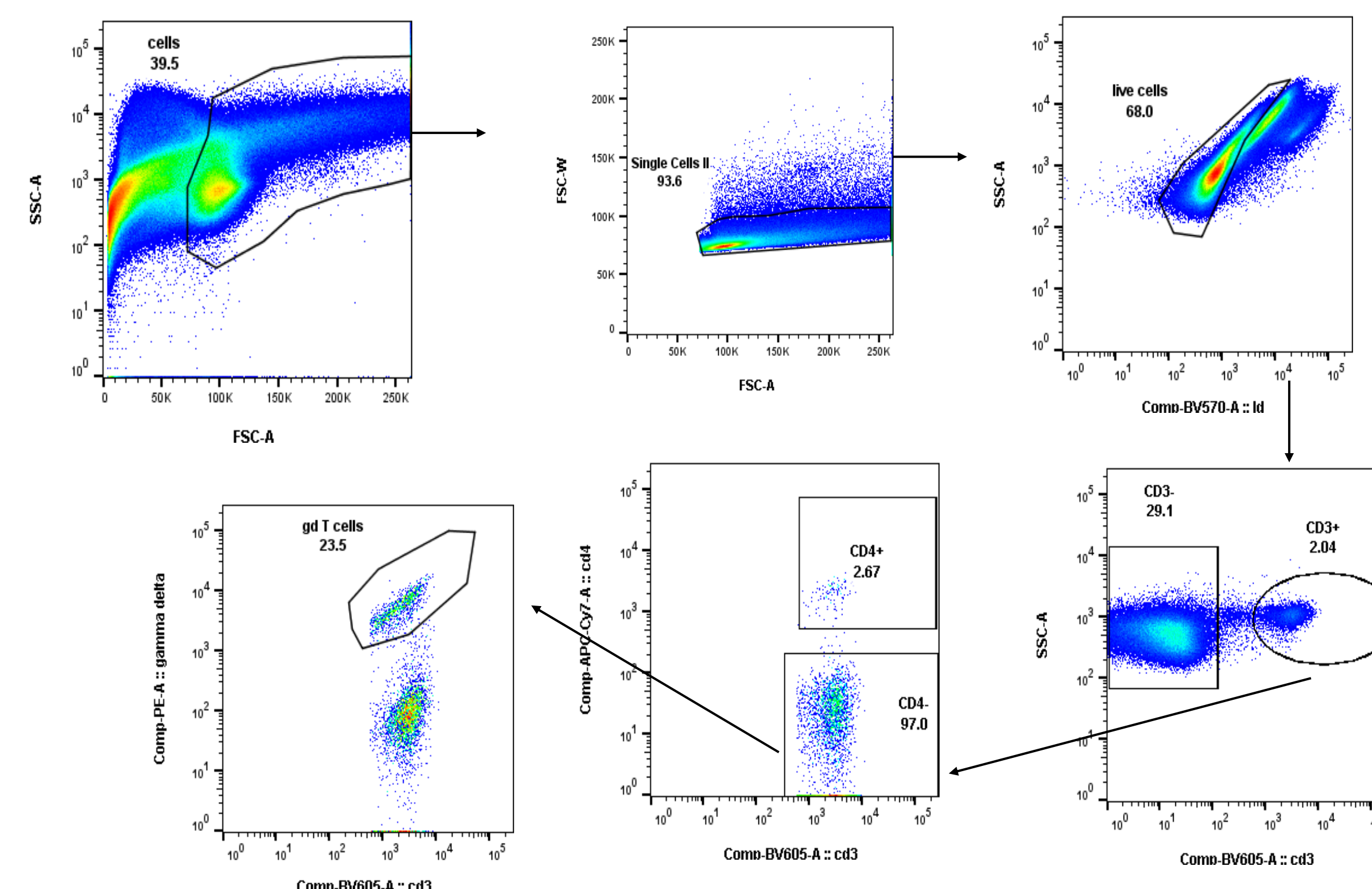
RNA from a set of site-specific biopsies was extracted with Qiagen RNeasy mini spin column and subsequently pooled to generate cDNA libraries with TakaraBio SMARTer kits and sequence on Illumina Hiseq4000. The raw files were quality-checked with FastQC, aligned to human genome (GRCh38) with STAR, and quantified for gene-level counts using RSEM method. DESeq2 was used for differential expression analysis ($\log_2\text{FoldChange} > 2$; $\text{FDR} < 0.01$). The Pathway enrichment analysis was done using KEGG package.

Sorted Cell RNA-seq | Sample Processing, Cell Sorting

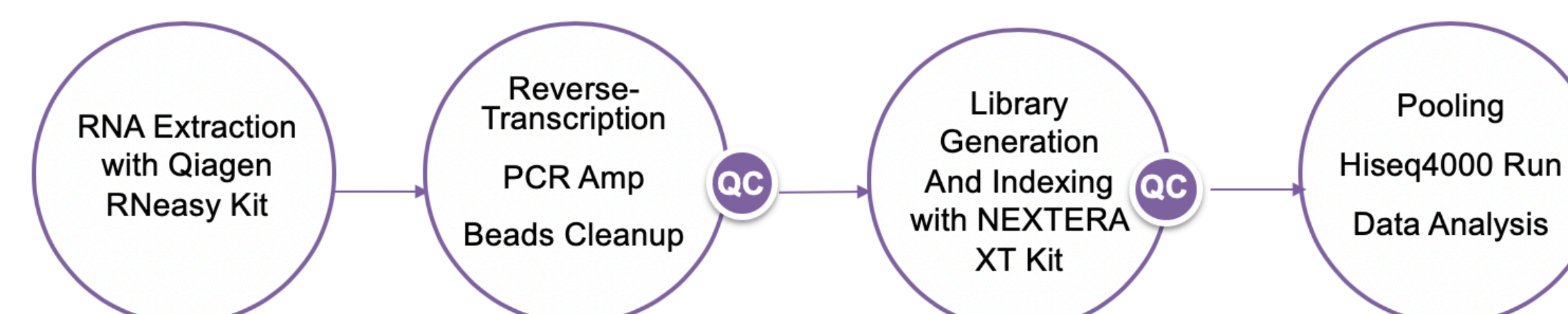
A set of five site-specific biopsies was pooled, homogenized and digested with Collagenase IV. The resulting cell suspension was stained with the

METHODS cont.

following fluorescent mAbs: gamma delta TCR-PE, CD4-APCCy7, CD3-BV605, and Live/Dead-Aqua dye. Gamma delta T cells were gated as shown below and bulk sorted in 100 μl PBS, pelleted, and kept frozen and kept frozen at -80°C until RNA extraction.



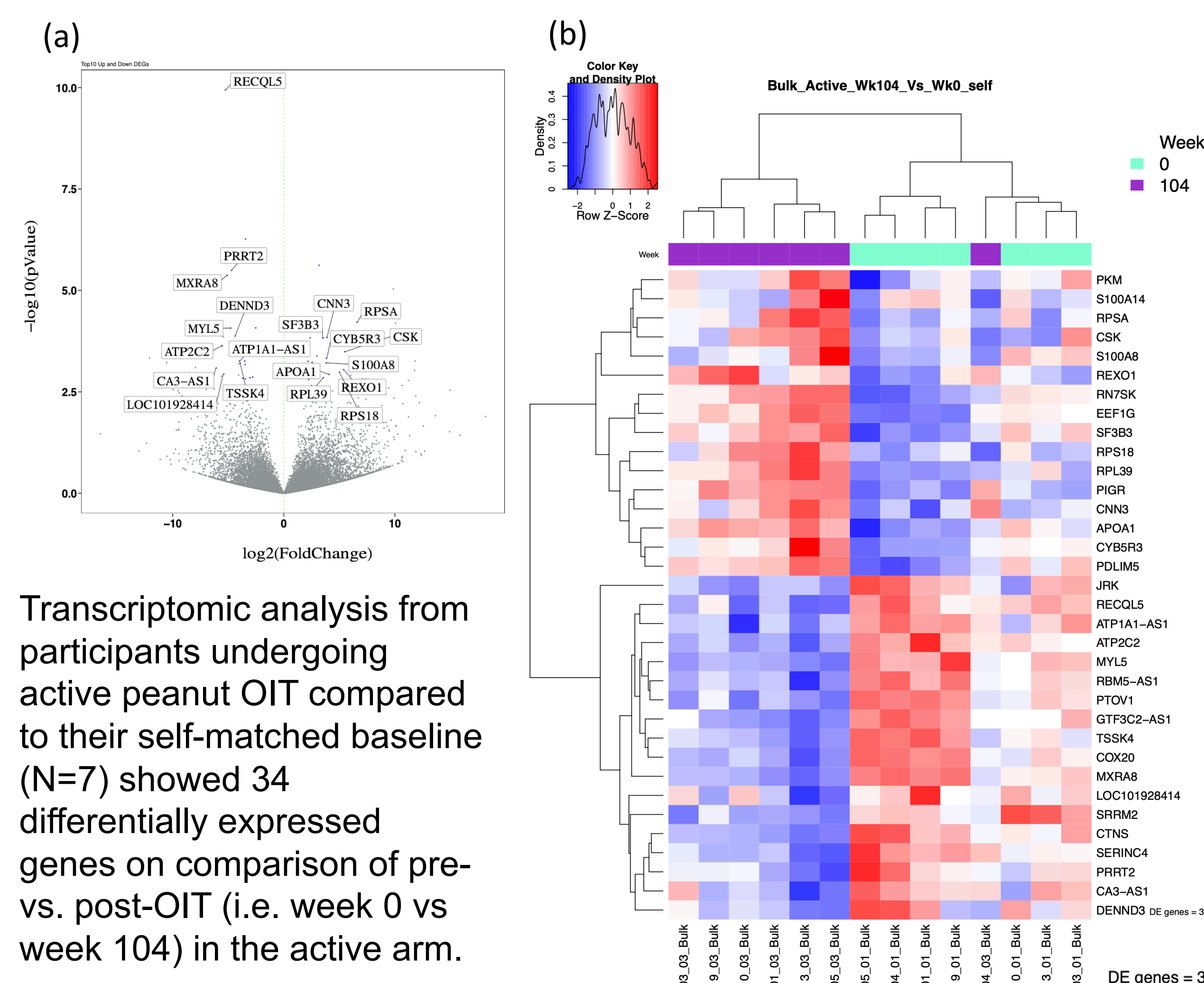
Sorted Cell RNA-seq | Workflow



RESULTS

Bulk Tissue RNA-seq | Differential Expression Analysis

Active arm | Week 0 Vs Week 104, N = 7

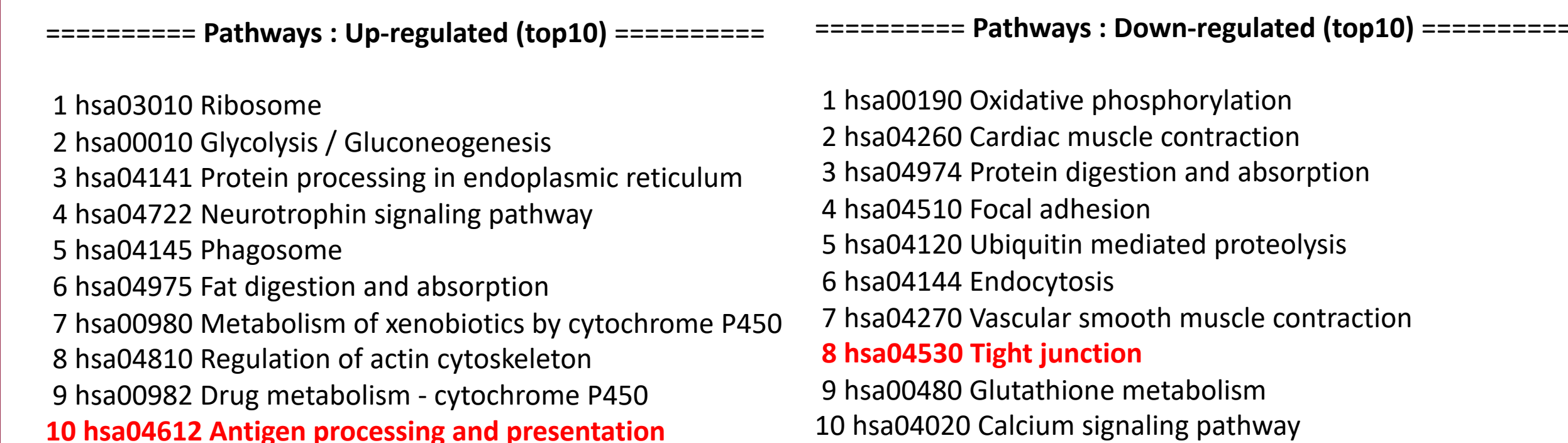


Transcriptomic analysis from participants undergoing active peanut OIT compared to their self-matched baseline (N=7) showed 34 differentially expressed genes on comparison of pre- vs. post-OIT (i.e. week 0 vs week 104) in the active arm.

RESULTS cont.

Bulk Tissue RNA-seq | Pathway Enrichment Analysis

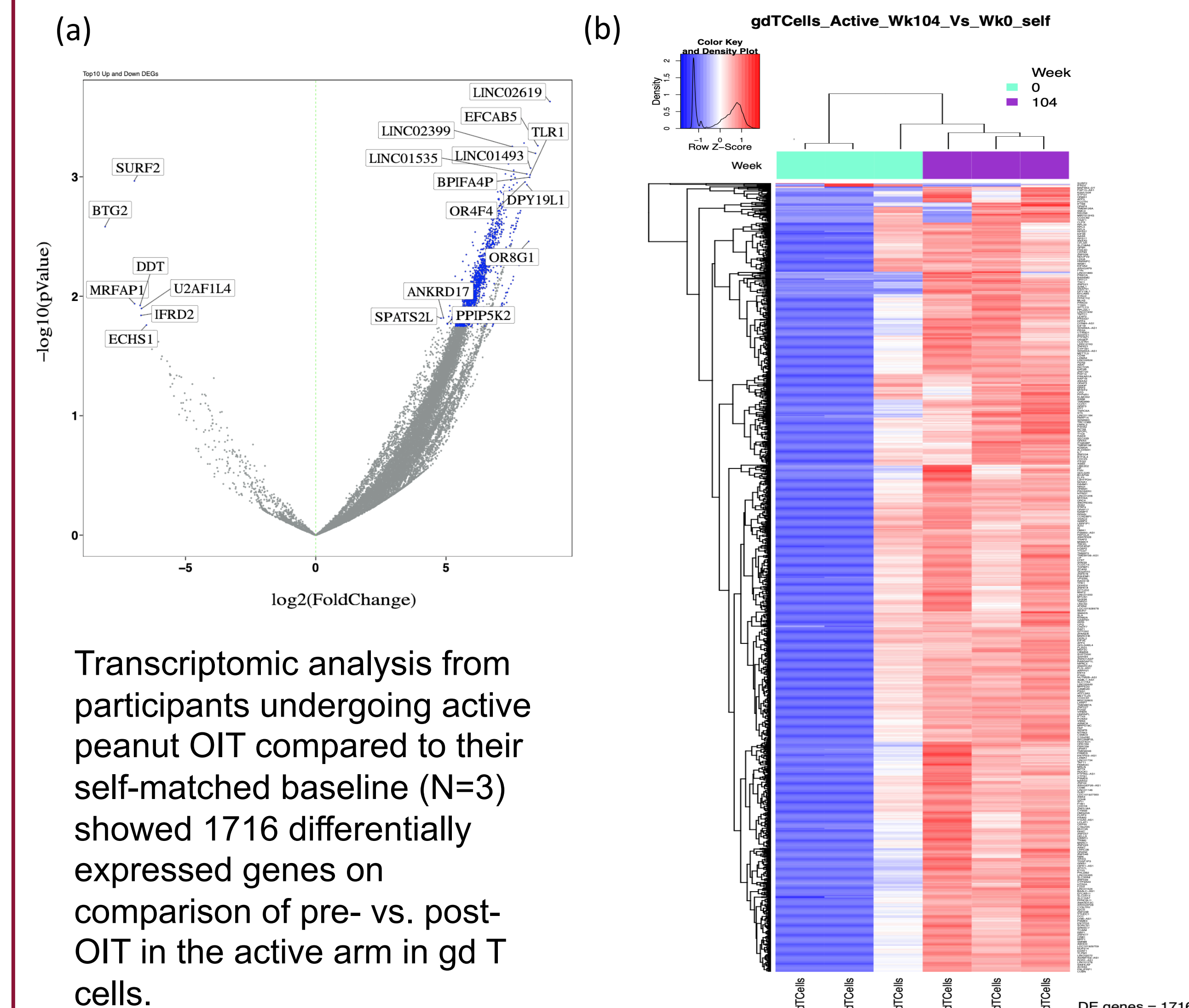
Active arm | Week 0 Vs Week 104, N = 7



KEGG pathway analysis revealed up-regulation of hsa04612 Antigen processing and presentation pathway and down-regulation of hsa04530 Tight junction pathway.

Sorted Cell RNA-seq | Differential Expression Analysis

Active arm | Week 0 Vs Week 104, N = 3



Transcriptomic analysis from participants undergoing active peanut OIT compared to their self-matched baseline (N=3) showed 1716 differentially expressed genes on comparison of pre- vs. post-OIT in the active arm in gd T cells.

ONGOING WORK

- Further analysis of RNA-seq results
- TCR-seq and Multiplex Ion Beam Imaging(MIBI)
- Confocal visualization of immune markers and tight junction markers on FFPE duodenum sections
- Secretory IgA level testing in stool samples

FUNDING

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